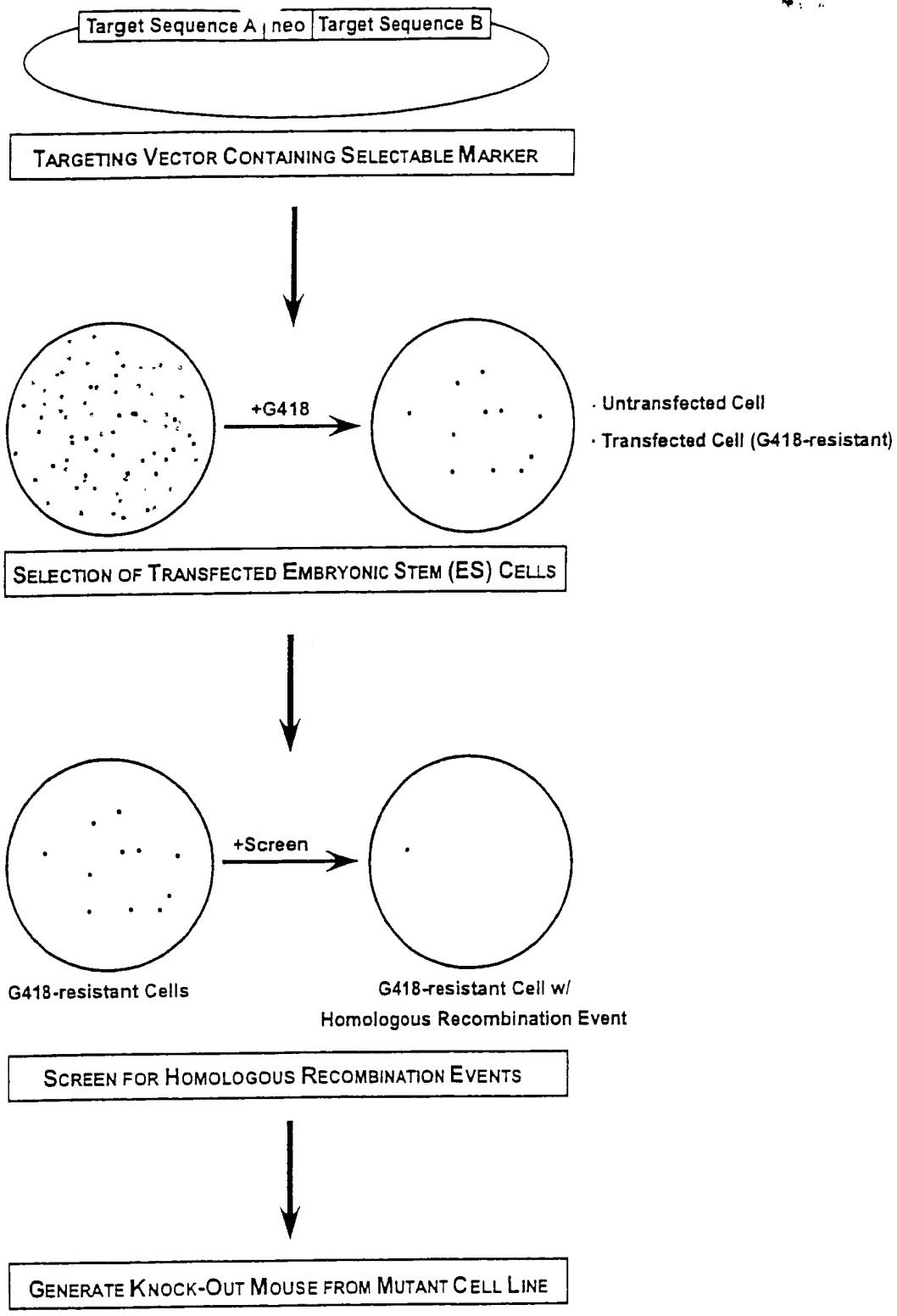
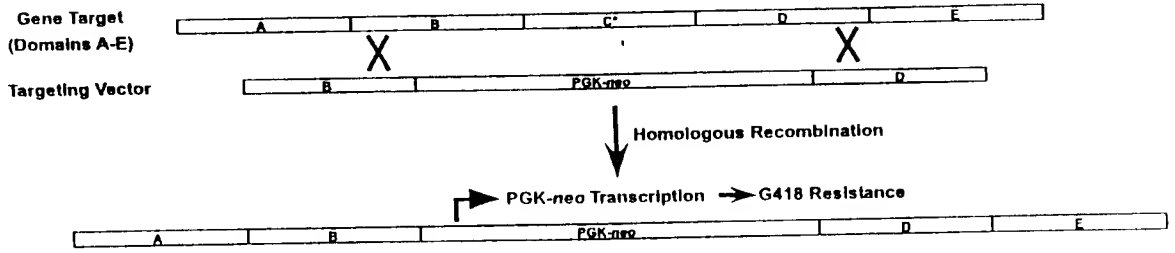
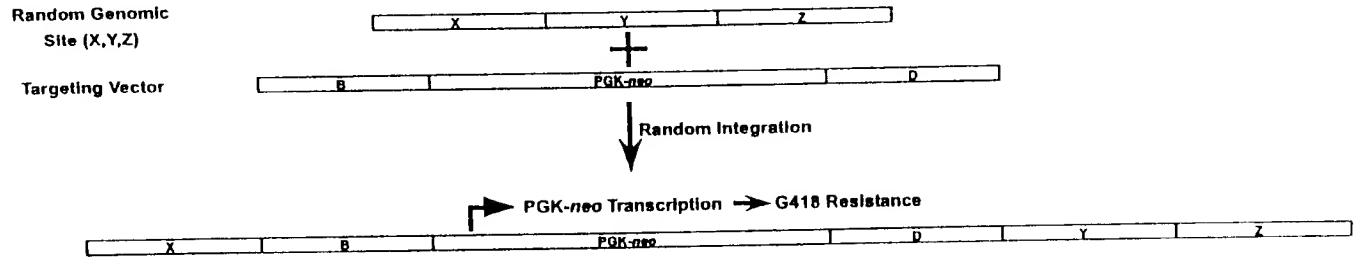

Target Sequence A
neo
Target Sequence B
TARGETING VECTOR CONTAINING SELECTABLE MARKER
+G418
· Untransfected Cell
· Transfected Cell (G418-resistant)
SELECTION OF TRANSFECTED EMBRYONIC STEM (ES) CELLS
+Screen
G418-resistant Cells
G418-resistant Cell w/
Homologous Recombination Event
SCREEN FOR HOMOLOGOUS RECOMBINATION EVENTS
GENERATE KNOCK-OUT MOUSE FROM MUTANT CELL LINE

FIGURE 1

A. Homologous Recombination: G418 Resistance, Targeting Vector Flanked by "A" and "E"



B. Random Integration: G418 Resistance, Targeting Vector Flanked by "X" and "Y"



DISTINGUISH EVENTS BY SCREENING MOLECULARLY (PCR & SOUTHERN)

FIGURE 2

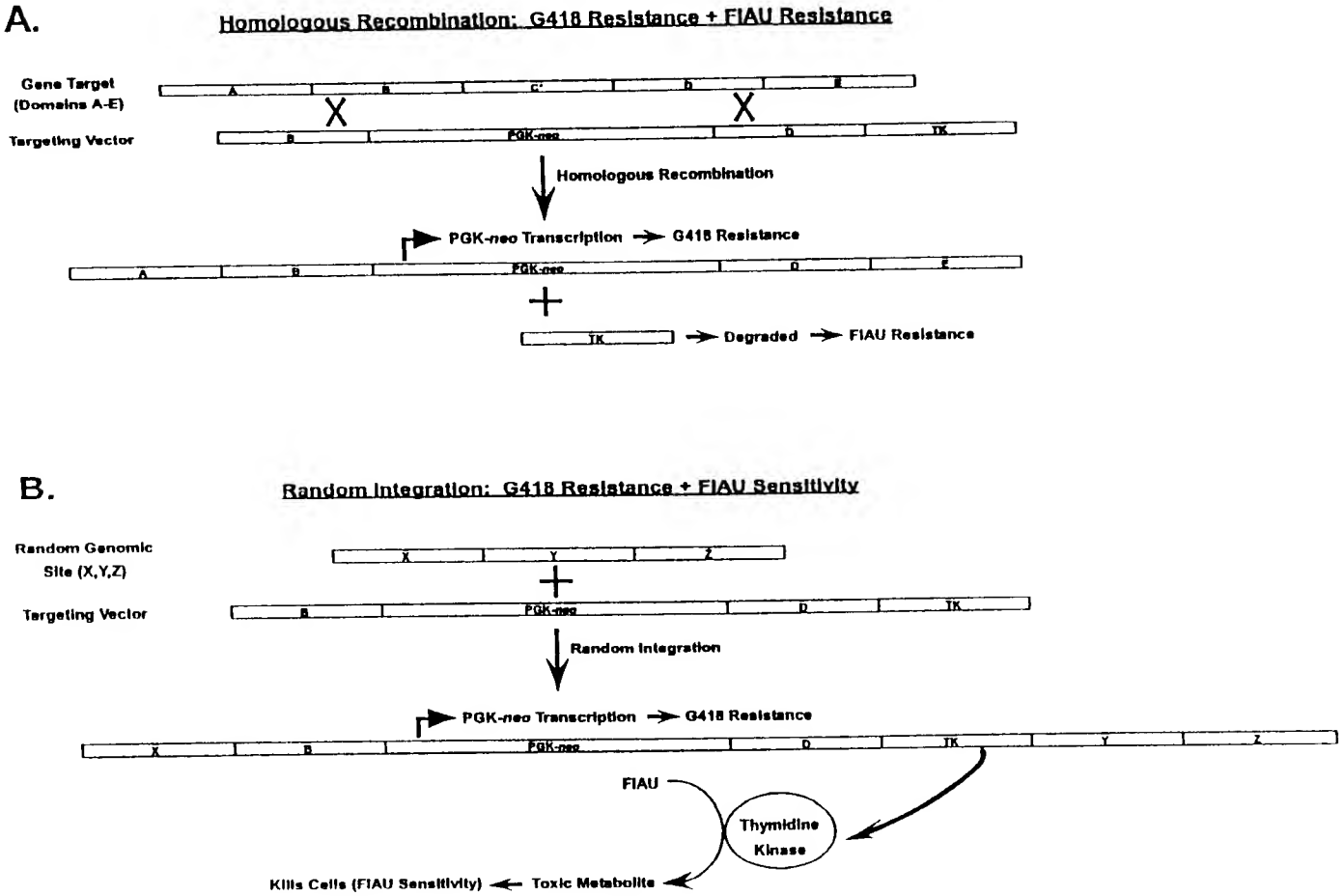

A.
Homologous Recombination: G418 Resistance + FIAU Resistance
Gene Target
(Domains A-E)
Targeting Vector
A
B
C'
D
E
PGK-neo
TK
Homologous Recombination
PGK-neo Transcription
G418 Resistance
Degraded
FIAU Resistance
B.
Random Integration: G418 Resistance + FIAU Sensitivity
Random Genomic
Site (X,Y,Z)
Targeting Vector
X
Y
Z
Random Integration
FIAU
Thymidine
Kinase
Kills Cells (FIAU Sensitivity)
Toxic Metabolite

FIGURE 3

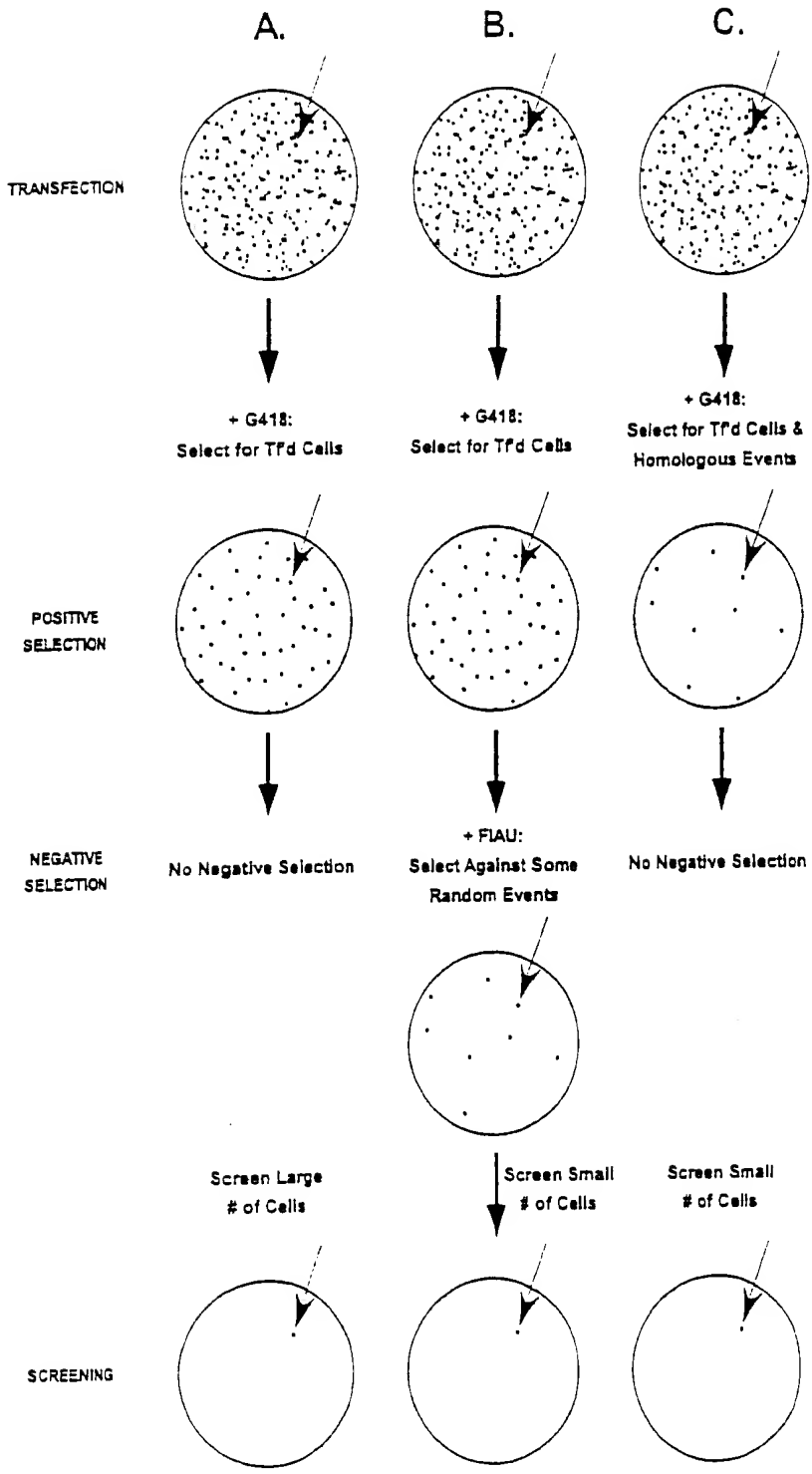

A.
B.
C.
TRANSFECTION
+ G418:
Select for Tfd Cells
+ G418:
Select for Tfd Cells
+ G418:
Select for Tfd Cells &
Homologous Events
POSITIVE
SELECTION
NEGATIVE
SELECTION
No Negative Selection
+ FIAU:
Select Against Some
Random Events
No Negative Selection
Screen Large
# of Cells
Screen Small
# of Cells
Screen Small
# of Cells
SCREENING

FIGURE 4

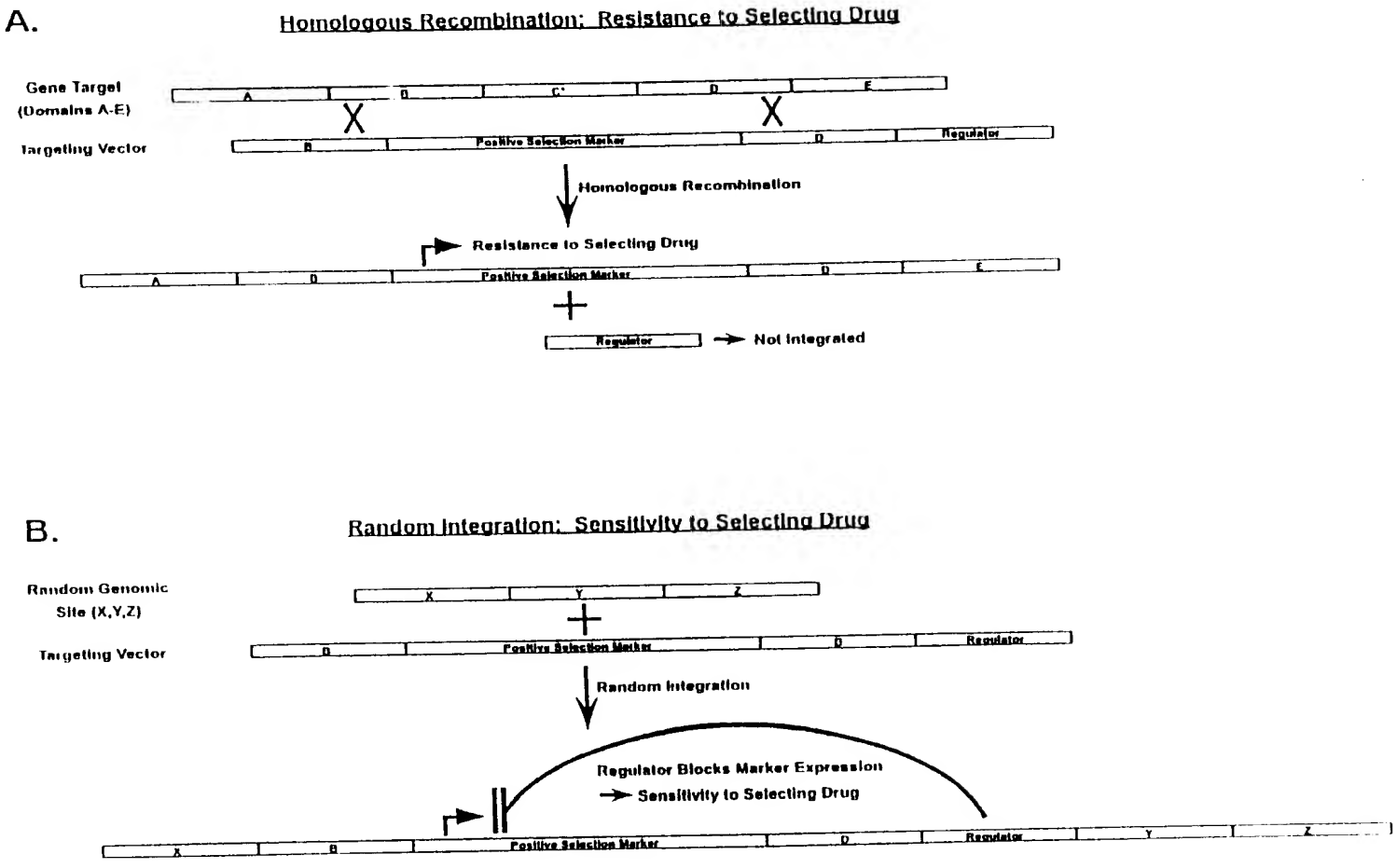

A.
Homologous Recombination; Resistance to Selecting Drug
Gene Target
(Domains A-E)
A
B
C
D
E
Targeting Vector
B
Positive Selection Marker
D
Regulator
Homologous Recombination
Resistance to Selecting Drug
A
B
Positive Selection Marker
D
E
Regulator
Not Integrated
B.
Random Integration; Sensitivity to Selecting Drug
Random Genomic
Site (X,Y,Z)
X
Y
Z
Targeting Vector
B
Positive Selection Marker
D
Regulator
Random Integration
Regulator Blocks Marker Expression
Sensitivity to Selecting Drug
X
B
Positive Selection Marker
D
Regulator
Y
Z

FIGURE 5

C3406:

GTTAACTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGC
TCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGT
GGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTCTCCAATGATGAGCACTTTTAAAG
TTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTT
GAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTG
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CTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCG
CTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAG
ATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGT
GCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTACCCCGGTTGATAATCAGAAAAGC
CCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAG
CTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTT
GGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCA
TCACCCAAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGG
AAAGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAAC
CACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATC
CCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAAT
CTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTG
GCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACA
TACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACC
GGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTAC
AGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGT
AATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACA
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ATAGGGACTTTCCATTGACGTCAATGGGAGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC
GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACA
TCTACGTATTAGTCATCGCTATTACCATGGTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCC
AATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGC
GAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG
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GGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGC
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GAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGGTGGCGGCAGGTGGGGGT
GCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGC
GAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAG
GCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCC
GCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGG
CTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGG
TTGTTGTGCTGTCTCATCATTTTGGCAAAGAATTCACCTGCCAGACCATGCCAAAAAAGAAGAGAAAGGTCATGAAACCAGTAACGTTA
TACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGA
AAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCG
TTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTG
GTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAA
CTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACAC
CCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTG
TTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGC
GGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGG
TTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATAC
GACGATACCGAAGACAGCTCATGTTATATCCCGCCGTCAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCG
CTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCA
ATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGAA
TTCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCC
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GTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTG
GCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCT
TATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCC
TTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCT
GCAGCCCAGCCCAAGCTCGGGGCCAGGTCGGCCGAGCGATCGCGAGAATTCGGCTTAAGTGAGTCGTATTACGGACTGGCCGTCGTTTT
ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAG
AGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTCGCTTGGTAATAAAGCCCGCTTCGGCGGGCT
TTTTTTT

FIGURE 6A

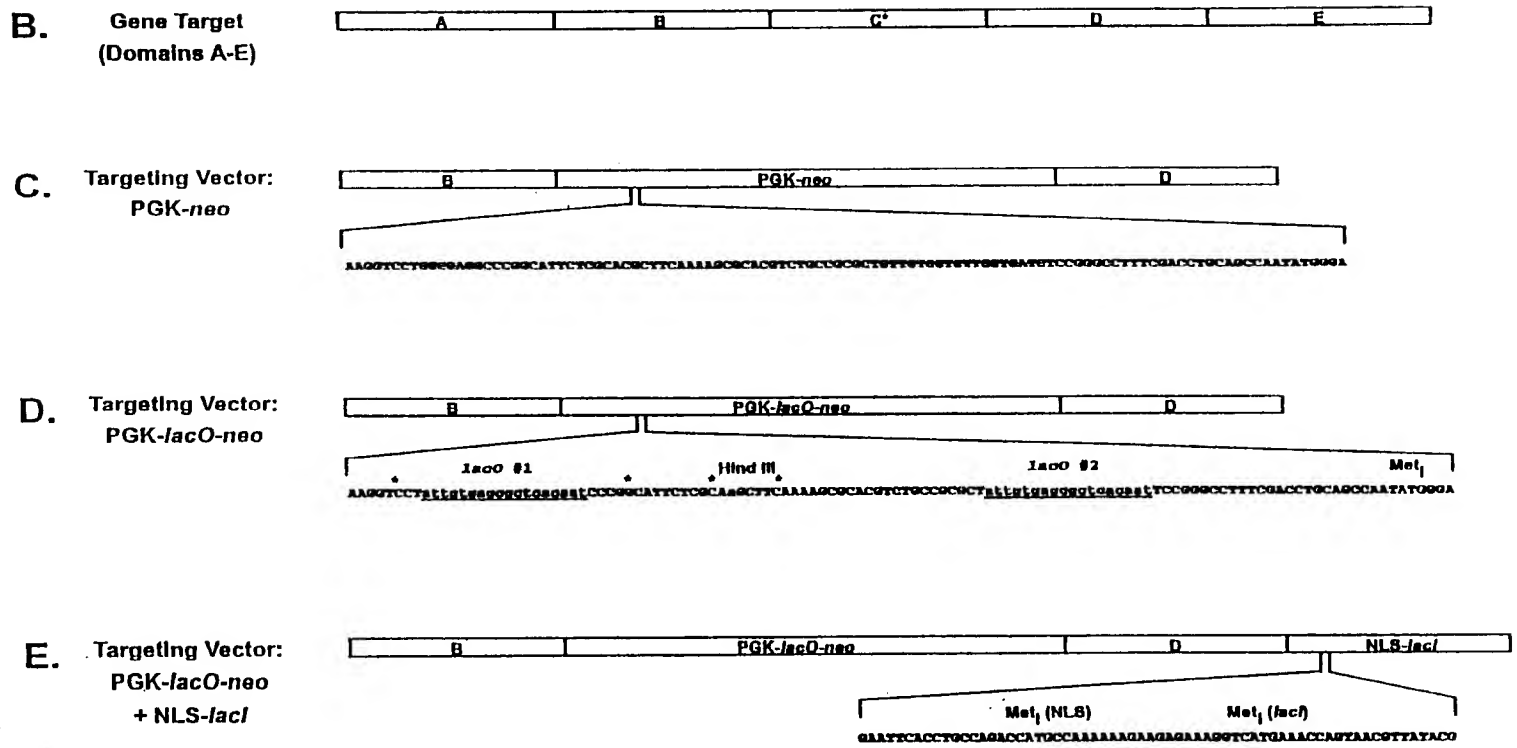
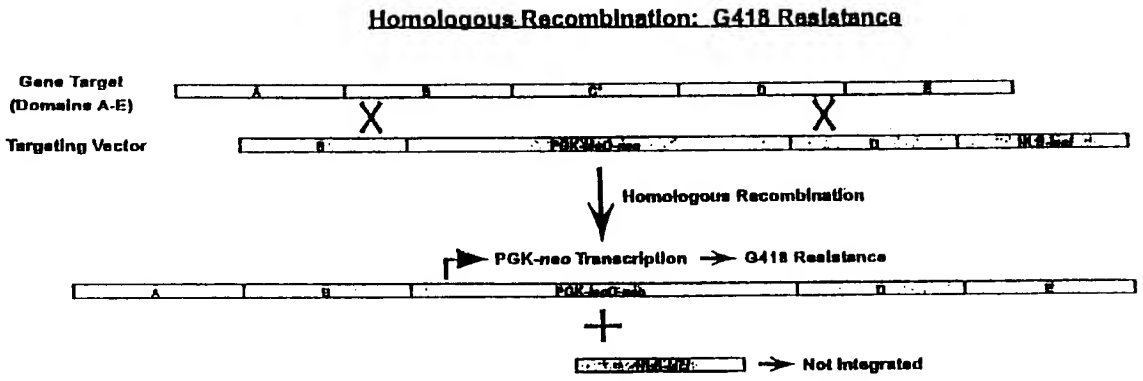

B.
Gene Target
(Domains A-E)
A
B
C*
D
E
C.
Targeting Vector:
PGK-neo
B
PGK-neo
D
D.
Targeting Vector:
PGK-lacO-neo
B
PGK-lacO-neo
D
lacO #1
Hind III
lacO #2
Met1
E.
Targeting Vector:
PGK-lacO-neo
+ NLS-lacI
B
PGK-lacO-neo
D
NLS-lacI
Met1 (NLS)
Met1 (lacI)

FIGURE 6 B-E

A.


Homologous Recombination: G418 Resistance
Gene Target
(Domains A-E)
Targeting Vector
Homologous Recombination
PGK-neo Transcription
G418 Resistance
Not Integrated

B.

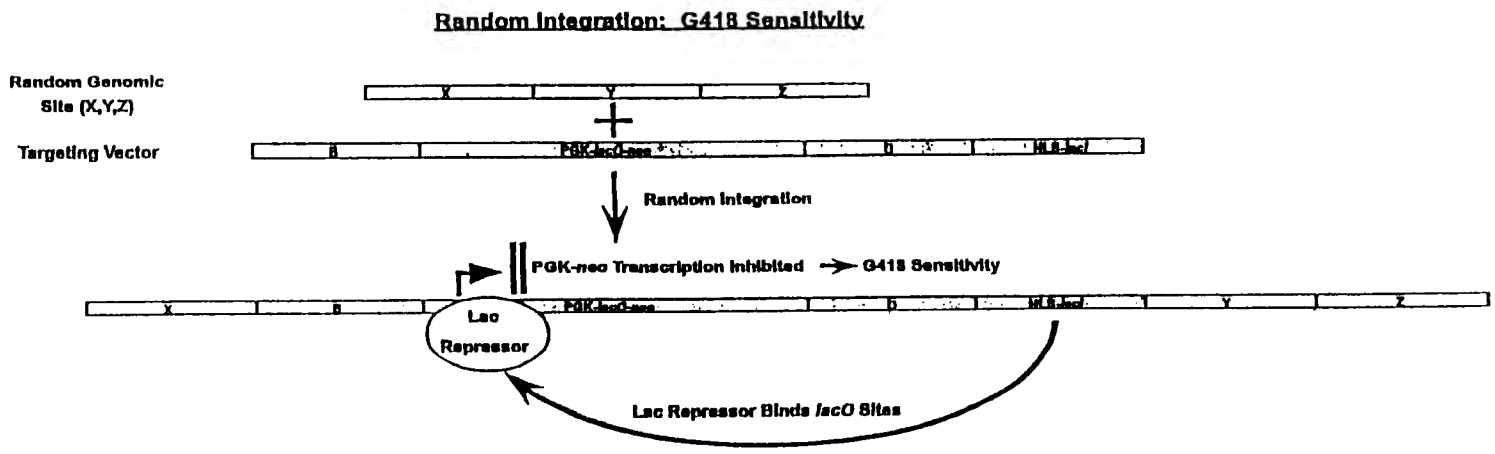

Random Integration: G418 Sensitivity
Random Genomic
Site (X,Y,Z)
Targeting Vector
Random Integration
PGK-neo Transcription Inhibited
G418 Sensitivity
Lac
Repressor
Lac Repressor Binds lacO Sites

FIGURE 7

Oligo #	Sequence (5' to 3')
10164	CGGAATTCACCTGCCAGACCATGCCAAAAAAGAAGAGAAAGGTCATGAAACCAGTAACGTTATACG
10165	CGGAATTCTCACTGCCCGCTTTCCAGTCG
10218	GCATTCTCGCAAGCTTCAAAAGCGCACGTCTGCCGCGCTATTGTGAGCGCTCACAATTCCGGGCCTTTCGACCTG
9959	TCATCAATTTCTGCAGAC
10219	TGCGCTTTTGAAGCTTGCGAGAATGCCGGGATTGTGAGCGCTCACAATAGGACCTTCGCGCCCGCC
4201	CAGGAAACAGCTATGAC

FIGURE 8

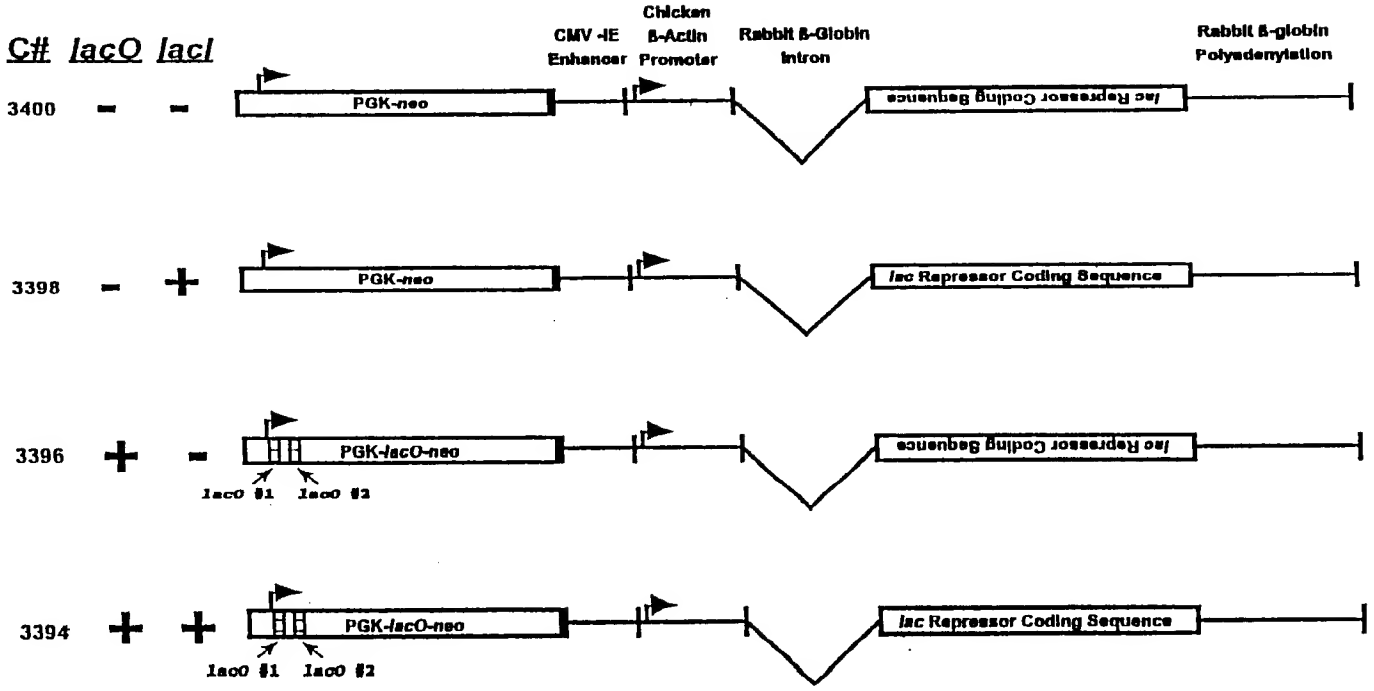

C# lacO lacI
CMV -IE Enhancer
Chicken β-Actin Promoter
Rabbit β-Globin Intron
Rabbit β-globin Polyadenylation
3400 - -
PGK-neo
lac Repressor Coding Sequence
3398 - +
PGK-neo
lac Repressor Coding Sequence
3396 + -
PGK-lacO-neo
lacO #1
lacO #2
lac Repressor Coding Sequence
3394 + +
PGK-lacO-neo
lacO #1
lacO #2
lac Repressor Coding Sequence

FIGURE 9

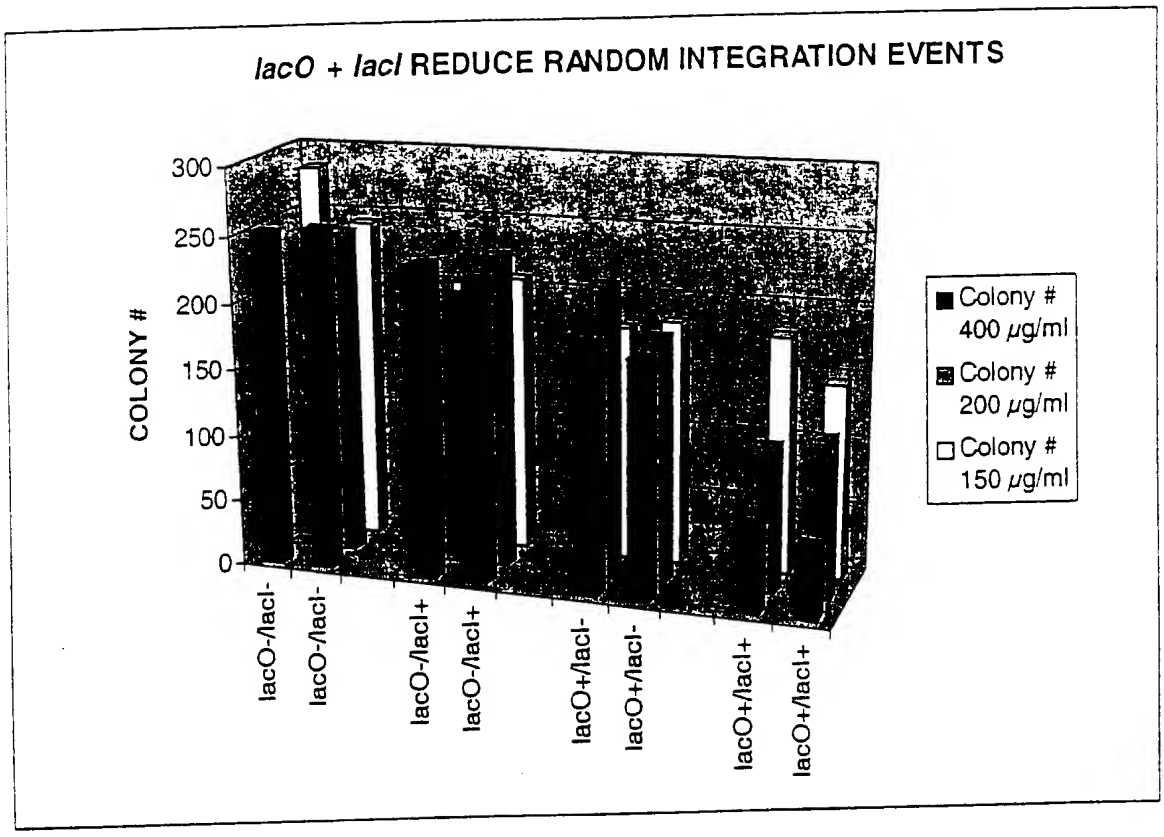

lacO + *lacI* REDUCE RANDOM INTEGRATION EVENTS
COLONY #
300
250
200
150
100
50
0
lacO-/lacI-
lacO-/lacI-
lacO-/lacI+
lacO-/lacI+
lacO+/lacI-
lacO+/lacI-
lacO+/lacI+
lacO+/lacI+
Colony # 400 μg/ml
Colony # 200 μg/ml
Colony # 150 μg/ml

FIGURE 10

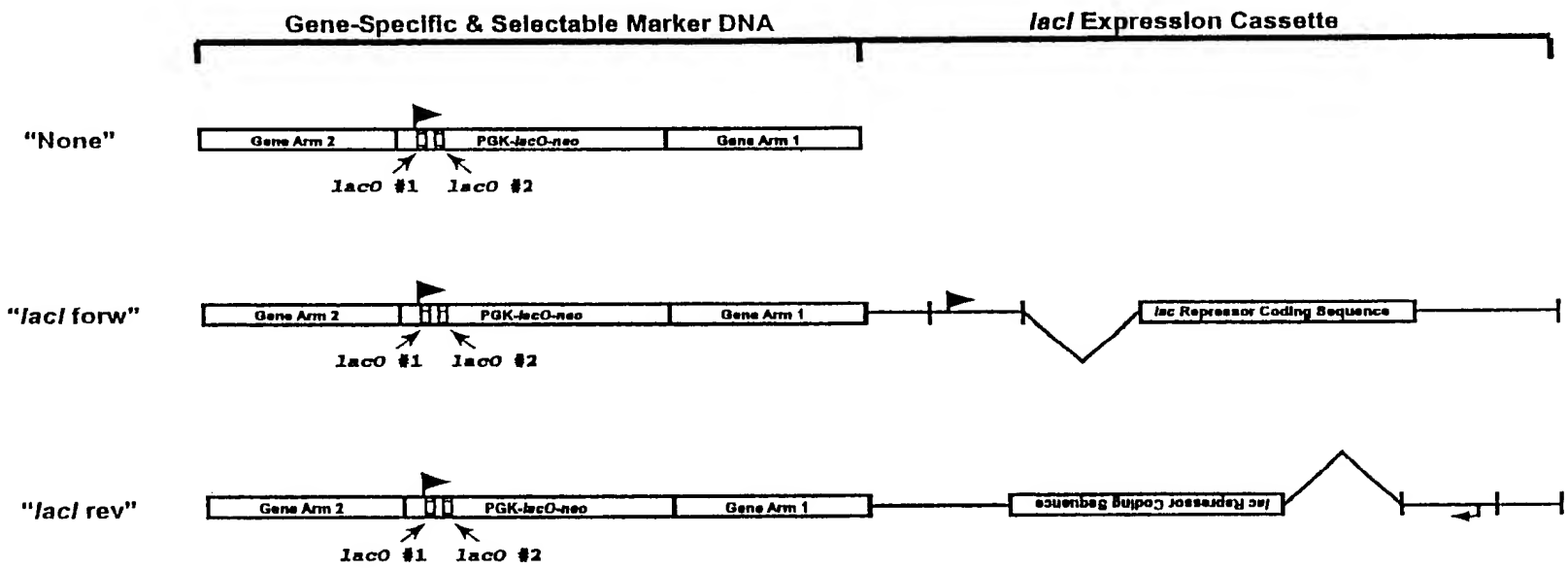

Gene-Specific & Selectable Marker DNA
lacI Expression Cassette
"None"
Gene Arm 2
PGK-lacO-neo
Gene Arm 1
lacO #1
lacO #2
"lacI forw"
Gene Arm 2
PGK-lacO-neo
Gene Arm 1
lac Repressor Coding Sequence
lacO #1
lacO #2
"lacI rev"
Gene Arm 2
PGK-lacO-neo
Gene Arm 1
lacO #1
lacO #2

FIGURE 11

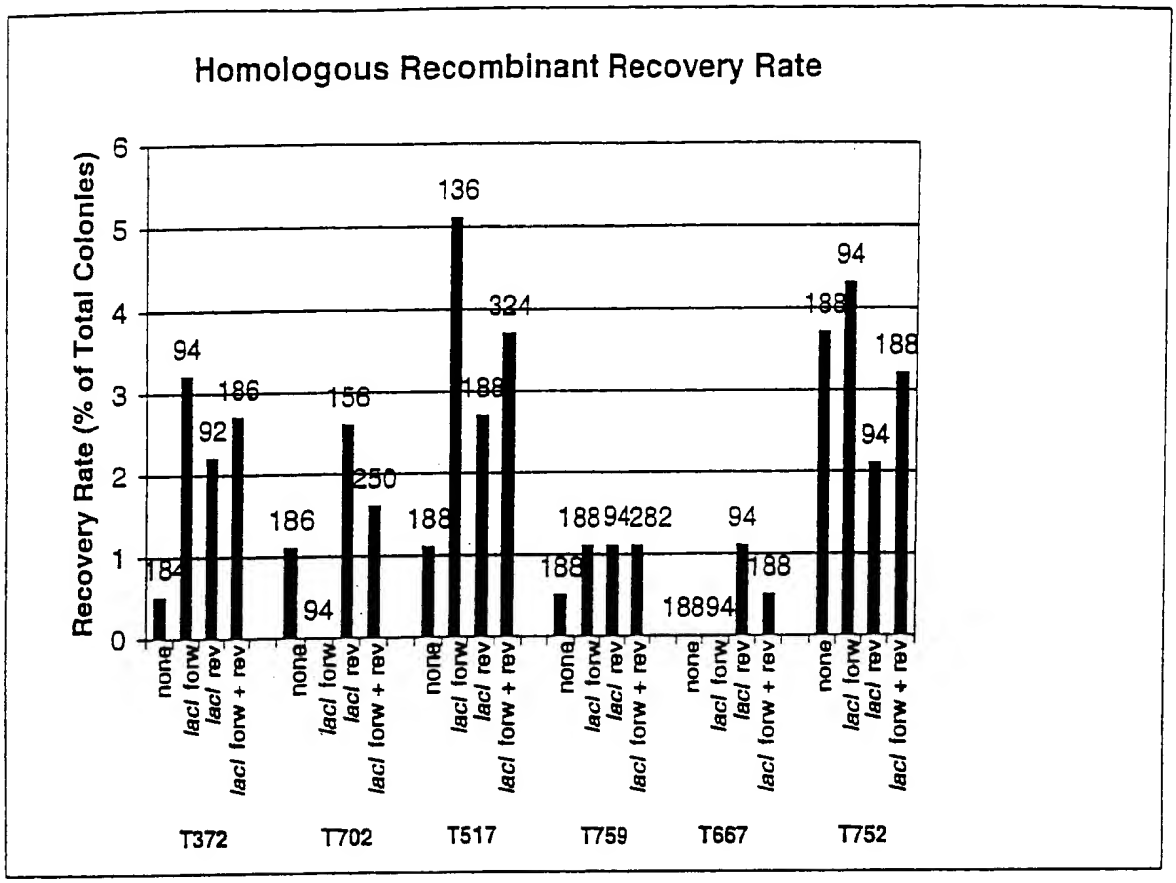

Homologous Recombinant Recovery Rate
Recovery Rate (% of Total Colonies)
0
1
2
3
4
5
6
184
94
92
186
186
94
156
250
188
136
188
324
188
188
94
282
188
188
94
94
188
188
94
188
none
lacI forw
lacI rev
lacI forw + rev
T372
T702
T517
T759
T667
T752

FIGURE 12

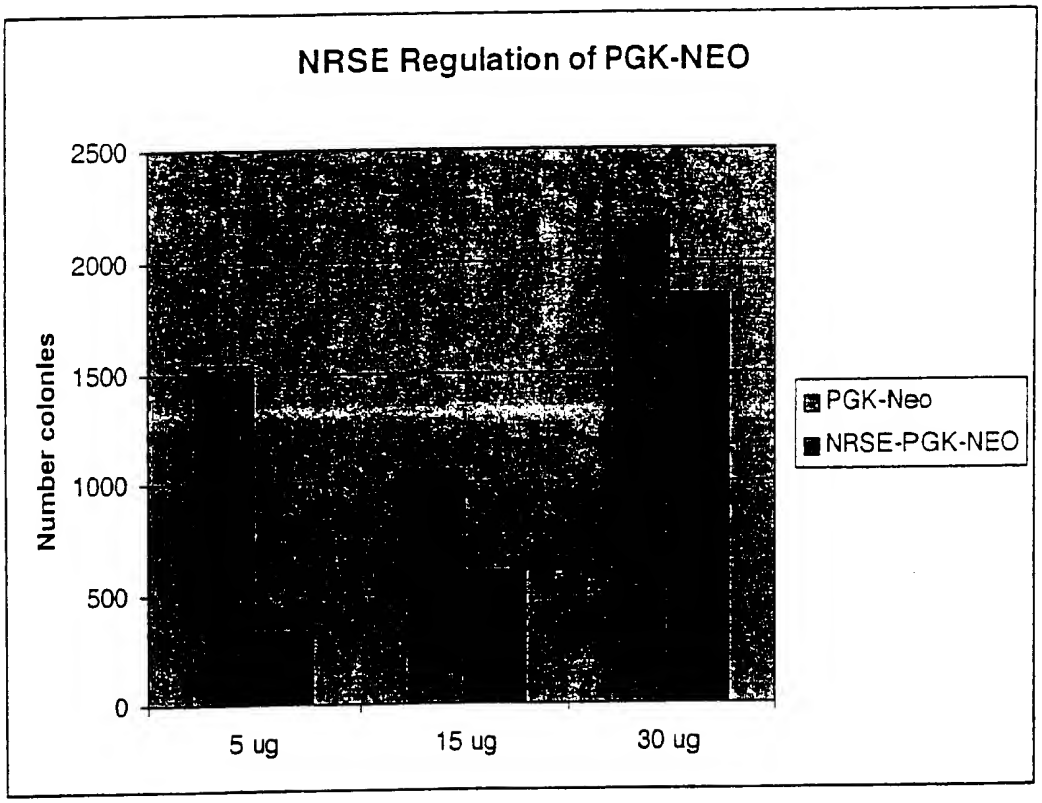

NRSE Regulation of PGK-NEO
Number colonies
2500
2000
1500
1000
500
0
5 ug
15 ug
30 ug
PGK-Neo
NRSE-PGK-NEO

FIGURE 13

GCGGCCGCGAGTCGACGAGGCCGGCCGATTAATTAAGGCTCgacattgattattgactag
ttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgt
tacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgac
gtcaataatgacgtATgttcccatagtaacgccaatagggactttccattgacgtcaatg
ggaggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaag
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GACCTTACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGTtcgaggtgagccccacgttctgcttcactctccccatctccccccccctccccacccc
caattttgtatttatttatttttttaattattttgtgcagcgatgggggcgggggggggggg
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gcggcggcagccaatcagagcggcgcgctccgaaagtttccttttatggcgaggcggcgg
cggcggcggccctataaaAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCG
CCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTA
CTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTT
TAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGC
CCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGC
CGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT
GCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGG
CTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGT
GGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACG
GCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCG
GGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCT
CGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAG
CCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTG
GCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGT
GCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTC
CCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACG
GGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGctctaGAGCCTCTGCTAACCA
TGTTCATGCCTTCTTCTTTTTCCTACAGctcctgggcaacgtgctggttgttgtgctgtc
tcatcattttggcaaagaattcGCCACCatggtgagcaagggcgaggagctgttcaccgg
ggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtc
cggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccac
cggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtg
cttcagccgctaccccgaccacatgaagcagcacgacttcttcaagtccgccatgcccga
aggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagacccgcgc
cgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgactt
caaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgt
ctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaa
catcgaggacggcagcgtgcagctcgccgaccactaccagcagaacaccccatcggcga
cggccccgtgctgctgcccgacaaccactacctgagcacccagtccgccctgagcaaaga
ccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgccgggatcac
tctcggcatggacgagctgtacaagtaaGAATTCACTCCTCAGGTGCAGGCTGCCTATCA
GAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCC
CTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA
AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAG
GACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGC
AACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATA
TGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGA
TTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTAC
ATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTC
TTCTCTTATGAAGATCcctcgacctgcagcccaagctCGGGGCCAGGTCGGCCGAGCGAT
CGCGAGAATTCGGCTTAAGTGAGTCGTATTACGGACTGGCCGTCGTTTTACAACGTCGTG
ACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
GCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGA

FIGURE 14A

ATGGCGAATGGCGCTTCGCTTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTTGGTTAA
CTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCT
AAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTG
CGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTG
AAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCC
TTGAGAGTTTTCGCCCCGAAGAACGTTCTCCAATGATGAGCACTTTTAAAGTTCTGCTAT
GTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACT
ATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCA
TGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACT
TACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGG
ATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACG
AGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCG
AACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTG
CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAG
CCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCC
GTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCAT
ATATACTTTAGATTGATTTACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGAT
TGTATAAGCAAATATTTAAATTGTAAACGTTAATATTTTGTTAAAATTCGCGTTAAATTT
TTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATC
AAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATT
AAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACT
ACGTGAACCATCACCCAAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCG
GAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCGAACGTGGCGAGAAAG
GAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTG
CGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTAAAAGGATCTA
GGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCA
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
CGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGA
TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA
TACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC
GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
ACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC
GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG
GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT
GGCCTTTTGCTGGCCTTTTGCTCACATGTAATGTGAGTTAGCTCACTCATTAGGCACCCC
AGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAAT
TTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAG

FIGURE 14B